

No reversion to single mating in a socially parasitic ant

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Abstract

Social Hymenoptera are ideal biological models for the study of the selective forces affecting the evolution of multiple mating (polyandry), because sister species can evolve different lifestyles and mating strategies. Single mating is predicted in workerless social parasites, because the key benefit of multiple mating in social insects, that is, the increase in genetic diversity among worker offspring, does not hold for workerless species. We compared the queen mating frequency between the ant *Plagiolepis pygmaea* and its derived social parasite *P. xene*. Previous studies showed that queens of the host *P. pygmaea* are obligately polyandrous. Here, pedigree analyses of mother–offspring combinations indicate that queens of the parasite *P. xene* did not revert to single mating; more than 50% of queens mated multiply, with 2–4 males. This result shows that reversal from multiple to single mating may be not selected in polyandrous social insect workerless parasites. We propose that such reversion does not occur when multiple mating is virtually cost free.

Introduction

Females of many animal species are polyandrous, mating with more than one male (Birkhead & Møller, 1998). However, in most instances, the adaptive significance of polyandry remains enigmatic because mating is assumed to be costly for females in terms of energy expenditure, exposure to predation or sexually transmitted parasites and pathogens (Daly, 1978; Chapman *et al.*, 1995, 2003). Compensating fitness benefits have been hypothesized to account for the widespread occurrence of multiple mating (reviewed in Eberhard, 1996; Hosken & Blankenborn, 1999; Arnqvist & Nilsson, 2000; Crozier & Fjerdingstad, 2001; Simmons, 2005), and over recent years, empirical evidence has accumulated in support of some of them in various taxa (Birkhead & Møller, 1998; Baer & Schmid-Hempel, 1999; Mattila & Seeley, 2007). Nevertheless, it remains difficult to ascertain the contribution of the different selective forces favouring the evolution and maintenance of polyandry. This stems primarily from the benefits and costs of multiple mating, which are difficult to quantify and are often similar in closely related species. A promising approach for assess-

ing the factors involved in the maintenance of polyandry is to compare the mating frequency between closely related species with different lifestyles (Sumner *et al.*, 2004). Changes in life history strategies may indeed profoundly alter the costs and benefits associated with mating and, hence, drive a shift in mating frequency.

Social Hymenoptera are ideal biological models to study the selective forces affecting the evolution of polyandry, because sister species can evolve different lifestyles and mating strategies. A remarkable example of such a variation in life history strategies concerns the transition to social parasitism, that is, the parasitic dependence of a social species on another free-living social species (Buschinger, 1990). Social parasitism is found in bees, wasps and in several ants. The most extreme form of social parasitism is permanent inquilinism, in which parasites have lost the worker caste and exploit the resources and workers of a host colony to raise their brood. Inquiline queens usually coexist with the host queen and produce only sexuals, which are reared by the host workers simultaneously with their own larvae. Studies in ants have shown that inquiline parasites are often the closest relatives of the host species (Buschinger, 1990; Huang & Dornhaus, 2008). Consistent with the impact of lifestyle on mating strategies, mating frequency comparison between the leaf-cutting ant *Acromyrmex echinator* and its recently derived social inquiline parasite *A. insinuator* has shown that

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host queens are highly polyandrous, while the social parasite queens are on average singly mated (Sumner *et al.*, 2004). Because queens of all free-living leaf-cutting ants are polyandrous (Villesen *et al.*, 2002), it is therefore likely that the common ancestor of both parasite and host probably had multiply mated queens. Thus, the change in lifestyle altered the benefits and costs of polyandry, so that the associated benefits were reduced for the parasite but specific to the free-living lifestyle. Reversal from multiple mating has been predicted to apply to other inquilines, because a key advantage of costly multiple mating in social Hymenoptera – i.e. the increased genetic diversity among worker offspring – does not hold for workerless social parasites (Sumner *et al.*, 2004). To date, however, whether workerless social parasites of other polyandrous social insects have also reverted to mating singly remains unknown.

We tested the possibility of reversal to single mating in the obligate workerless social parasite ant *Plagiolepis xene*. This species is derived recently from its host *P. pygmaea*, being highly specialized to it (Trontti, 2006). In all free-living *Plagiolepis* ants studied so far, queens are facultatively or obligately multiply mated (*sensu* Boomsma *et al.*, 2009; strict monandry: all queens being singly mated; facultative polyandry: $\geq 50\%$ of queens singly mated and a minority mated with 2–5 males; obligate polyandry: usually ≥ 2 and often ≥ 5 matings per queen), suggesting that multiple mating is ancestral to the genus (Trontti *et al.*, 2007; Thurin & Aron, 2010). Using microsatellite marker loci, we determined the queen mating frequency in *P. xene*. Mating frequency in the parasite was then compared with its free-living host *Plagiolepis pygmaea* where queens are obligately multiply mated, with more than 80% of the queens experiencing ≥ 2 mating (absolute mating frequency $M_p = 2.89$; effective queen mating frequency $M_{e,p} = 1.93$; Trontti *et al.*, 2007).

Materials and methods

Study species

Plagiolepis xene is rare and extremely difficult to find; fewer than 1% of the host colonies are parasitized (Passera, 1964). Field observations and genetic analyses showed that, in both the host and the parasite, colonies contain multiple queens (polygyny), mating takes place inside the nest among related individuals and populations are highly inbred (Passera, 1969; Trontti *et al.*, 2005, 2006, 2007; Thurin & Aron, 2009). Sexuals of the parasite are significantly smaller than host sexuals (Aron *et al.*, 1999), and *P. xene* males are wingless. Parasite dispersal occurs when host colonies reproduce by budding, a process whereby newly mated queens leave the mother nest with a worker force and found a new colony nearby (Passera *et al.*, 2001). Independent foundation (i.e. without the help of a worker force) is very limited in *P. xene*, in which case a single or at most

a few mated queens fly away and infiltrate host colonies from the same or other populations (Trontti *et al.*, 2006).

Sampling and queen mating frequency analyses

Thirty-one parasitized *P. pygmaea* colonies were collected from two populations (Tarabel and Bordeneuve) distant 15 km apart, in Southern France, in March 2007, 2008 and 2009. From these collections, we settled 231 experimental nests, each containing a single parasite queen, two host queens and about 300 host workers. The ants were fed honey and water, and kept at 27 ± 1 °C. The sexual offspring produced by the parasite were removed from the experimental nests and stored at -80 °C for subsequent genetic analyses.

We estimated the minimum number of fathers contributing to the progeny of each *P. xene* queen by reconstructing each paternal genotype from mother–female offspring allele combinations. (Males arise from unfertilized, haploid eggs) The genotype of the queens and their female offspring was determined at eight microsatellite loci (Trontti *et al.*, 2003). Nuclear DNA was isolated from the thorax. Individual ant DNA was extracted by incubating for 120 min in 40- μ L Chelex (Bio-Rad, Hercules, CA, USA) at 85 °C. Samples were centrifuged for 30 s at 10 000 *g*, and 1.5 μ L of the supernatant was amplified by PCR following the fluorescent analysis protocols described in Trontti *et al.* (2003), using a PTC-200 thermal cycler and Taq Gold polymerase (Fermentas, St Leon-Rot, Germany). Amplified fluorescent fragments were visualized using an automated ABI Prism 3100 sequencer.

The number of distinct paternal genotypes inferred per experimental nest provides the minimal number of mates of each *P. xene* queen (M_p). Because the males may contribute unequally to the offspring, we estimated the effective mating frequency ($M_{e,p}$) following Nielsen *et al.* (2003),

$$M_{e,p} = \frac{(n-1)^2}{\sum_{i=1}^k [p_i^2(n+1)(n-2) + 3 - n]}$$

where n is the total number of offspring of a queen, k is the number of male mates and p is the proportional contribution to the brood of the i th male. The effective number of patrines equals the absolute mating frequency (M_p) when all males contribute equally.

We determined the probability of nonsampling of a father contributing to ten per cent of the progeny owing to reduced offspring sampling, using the equation of Foster *et al.* (1999),

$$P_{\text{non-sampling}} = (1-p)^n$$

where p is the father's contribution to the progeny and n the number of sampled offspring. We also calculated the probability of nondetection of additional patrines

attributed to two fathers sharing the same alleles at all loci studied using the equation:

$$P_{\text{non-detection}} = \frac{\sum_k \Pi_j \sum_i f_{ijk}^2}{n} \quad (1)$$

where f_{ij} is the nest k level frequency of allele i at locus j and n is the number of nests (Boomsma & Ratnieks, 1996). Inbreeding in *P. xene* populations further increases the probability of such nondetection errors.

Unbiased comparison of queen mating frequency between the host and the parasite

Given the parasite's low genetic diversity (see Results), there was a high probability that some matings remained undetected, and hence, it is likely that the queen mating frequency in *P. xene* has been underestimated. The estimated allelic richness for the eight loci typed was indeed lower in the parasite than that previously reported for the host by Trontti *et al.* (2007). This could bias our comparison of queen mating frequency between the host and the parasite. To take this potential bias into account, we artificially reduced the diversity of the *P. pygmaea* parent-offspring data set (data from Trontti *et al.*, 2007) to mirror the level of genetic diversity found in the *P. xene* parasite data set. More specifically, at each locus, we randomly merged existing alleles into new alleles, until we reached the same allelic richness and similar allele frequencies ($\pm 5\%$) than those found in the parasite data set. This transformed data set was used to estimate a standardized value of the effective queen-

mating frequency for the host. When more than one way to merge alleles was possible, we considered the mean value obtained from the different combinations.

Results

Six of the 231 *P. xene* reared queens died during the experiment. Consistent with the extremely low productivity of the species (Passera, 1964), only 21 queens (< 10%) originating from 15 colonies produced at least two female offspring (five nests reared two offspring, six nests three offspring, six nests four offspring, two nests five offspring, one nest six offspring and one nest 15 offspring; Fig. 1). The genetic diversity, measured as allelic richness, in the two sampled populations was very low. The number of alleles per loci ranged from one to five in Tarabel (mean number of alleles \pm SD = 2.75 ± 0.45 , $n = 53$ queens) and from 2 to 4 in Bordeneuve (mean number of alleles \pm SD = 2.87 ± 0.35 , $n = 56$ queens).

Pedigree analyses from mother-offspring combinations were consistent with half *P. xene* queens mating multiply (Fig. 1). Eleven queens (52%) were found at least doubly mated and one queen mated with up to four males. The harmonic mean effective number of mating per queen \pm SE in the inquiline *P. xene* was $M_{e,p} = 1.30 \pm 0.21$. This value was significantly lower than in its free-living host *P. pygmaea*, where queens mate with 1–6 males and $M_{e,p} \pm$ SE = 1.93 ± 0.20 (Trontti *et al.*, 2007) (two-tailed t -test, $t = 2.15$, $P = 0.04$).

Our estimate of queen-mating frequency in *P. xene* is highly conservative, as both the probabilities of

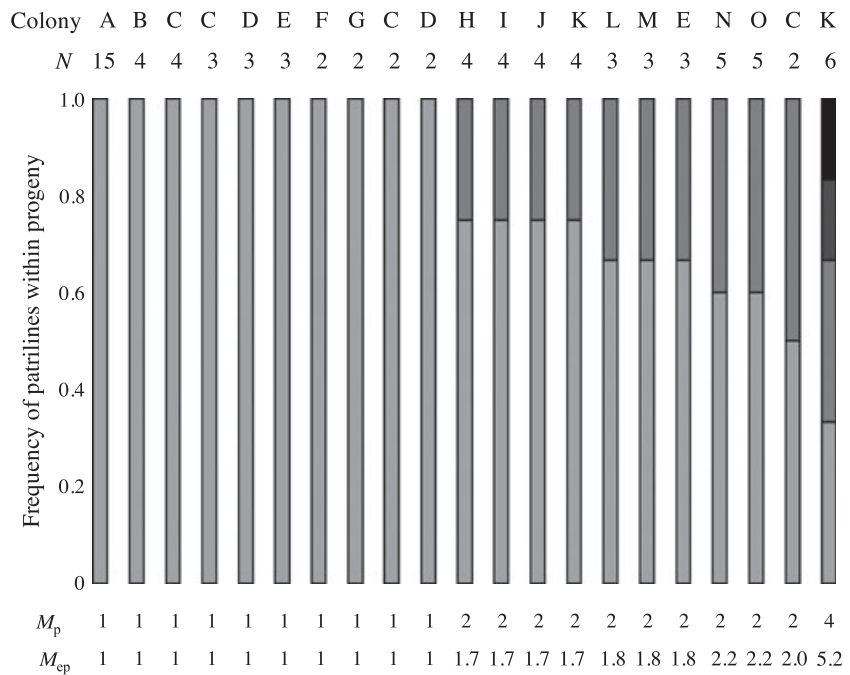


Fig. 1 Observed frequency distribution of patriline (offspring sired by different males) in the social parasite *Plagiolepis xene* ($n = 21$ experimental nests from 15 colonies noted A–O), as derived from genetic analysis of the progeny of single queens. Patriline frequencies are shown by alternate shading patterns. The estimated absolute number of mating (M_p) and effective mating frequency ($M_{e,p}$) for each experimental nest are given below the bars. The number of female offspring produced and typed in each experimental nest (N) is indicated above each bar.

nonsampling (mean \pm SE: 0.68 ± 0.03) and nondetection errors (0.28 ± 0.10) are high. We compared the effective queen-mating frequency between both species by taking into account the probability of errors and simulating the same allelic richness and similar allele frequencies in the host and the parasite. The corrected mating frequencies were not significantly different thereafter ($M_{e,p}^* = 2.23 \pm 0.17$ in *P. pygmaea* vs. 2.55 ± 0.41 in *P. xene*; two-tailed *t*-test, $t = 0.67$, $P = 0.51$).

Discussion

Reversion to single mating is predicted to occur in workerless social parasites owing to life history strategy changes associated with parasitism, which are expected to profoundly alter the costs and benefits of polyandry and, hence, drive shift in mating frequency (Sumner *et al.*, 2004). Our data are not consistent with this hypothesis. They show that more than 50% of parasitic queens mate multiply, with 2–4 males. Moreover, the proportion of multiply mated queens is similar to that reported in the free-living host *P. pygmaea* (Trontti *et al.*, 2007). Thus, *P. xene* must be considered as polyandrous and falls into the intermediate mating system class of facultative to obligate polyandry (according to Boomsma *et al.*, 2009). Mating frequency in the parasite is most likely underestimated because of a high probability of nondetection and nonsampling. First, *P. xene* populations are established by a few individuals and experience a strong founder effect (Trontti *et al.*, 2006). In addition, new populations are very small and mating takes place between close relatives (Trontti *et al.*, 2006). Both the founder effect and inbreeding greatly reduce allelic richness in populations (Charlesworth & Wright, 2001; Charlesworth, 2003), thereby increasing the probability that additional matings remain undetected because of two males bearing the same alleles at all eight loci studied (probability of nondetection: 28% in *P. xene* vs. 2% in the host *P. pygmaea*). In line with this hypothesis, no difference was found in the effective mating frequency between *P. pygmaea* and *P. xene* queens when the genetic diversity in the host was reduced to that found in the parasite. This suggests that there might even be no reduction in the queen mating frequency of the parasite. Secondly, *P. xene* queens are weak layers and produce few offspring under laboratory conditions (Passera, 1969; this study); this severely increases the probability of nonsampling (68% in *P. xene* vs. 27% in the host *P. pygmaea*).

Several fitness benefits have been hypothesized to account for the evolution and maintenance of multiple mating in social Hymenoptera (reviewed in Crozier & Page, 1985; Crozier & Fjerdingstad, 2001). Five of these apply equally to social parasites and free-living species. Polyandry allows (i) increased sperm competition to produce offspring with higher reproductive output, (ii) avoidance of mating with a single male to avoid genetic

incompatibility risks, (iii) production of genetically variable daughter queens to enhance the chance that some will survive and (iv) reducing the variance in the production of sterile diploid males, which arises when there is homozygosity at the sex locus. The high level of inbreeding reported in both *P. xene* and *P. pygmaea* (Trontti *et al.*, 2005, 2006) should greatly reduce the benefits associated with these hypotheses. Detailed genetic analyses previously showed that the queen's male mates are highly related to the queen and to each other and that polyandry in *P. pygmaea* does not alleviate the loss of genetic variation caused by fixation of alleles owing to inbreeding and genetic drift (Trontti *et al.*, 2007). Multiple mating also increases the probability of obtaining an unrelated mate and may increase the variance in inbreeding among offspring. This could favour polyandry in *Plagiolepis*, where outbreeding is rare, especially if outbred offspring confer a fitness advantage. However, when given the choice, *P. pygmaea* virgin queens mate preferentially with related rather than with unrelated males (Thurin & Aron, 2009). Evidence that the production of diploid males is not a strong selective force promoting multiple mating in *Plagiolepis* is also supported by the fact that no diploid males were found despite the high level of inbreeding reported in all *P. pygmaea* and *P. xene* populations (Trontti *et al.*, 2005, 2006; Thurin & Aron, 2008). Although one may not completely exclude the possibility that diploid males are eliminated by workers during development in the host, as documented in the honeybee (Woyke, 1963, 1980; Santomauro *et al.*, 2004), this explanation seems unlikely for the parasite. Social parasites have repeatedly been able to overcome the odour-based nestmate discrimination code of their hosts to achieve social integration in host colonies (Lenoir *et al.*, 2001; D'Ettoire *et al.*, 2002). Previous studies showed that the social parasite *P. xene* brood escape recognition by host workers and develop into sexuals even when the *P. pygmaea* host actively prevents the production of its own sexuals (Aron *et al.*, 1999, 2004). Thus, if *P. xene* queens produced diploid males, some should have been detected. (v) According to the *sexually selected sperm* hypothesis (Keller & Reeve, 1995), females mating multiply increase the probability that their eggs are fertilized by a male with high fertilization efficiency and that their sons will inherit the genes specifying for highly competitive sperm. This hypothesis has been challenged by Pizzari & Birkhead (2002) because many male efficient fertilizing traits are heritable through sex-biased mechanisms and may not necessarily increase female fitness. More importantly, it seems unlikely to be significant in the maintenance of Hymenoptera polyandry, because males develop from unfertilized eggs. Three other benefits of multiple mating are specific to free-living social insects, but do not apply to workerless species: (vi) the production of a genetically diverse worker force to enhance colony resistance to parasites and pathogens or colony

productivity, (vii) a reduction in within-colony relatedness asymmetries to lessen social conflicts and (viii) increase sperm supply storage to maintain large and long-lived colonies.

On the other hand, a potential benefit that may justify the maintenance of polyandry in *P. xene* is (ix) mating for convenience to avoid confrontations with males (Thornhill & Alcock, 1983). Convenience polyandry has been proposed to account for multiple mating in various ant species (Chapuisat, 1998; Pedersen & Boomsma, 1999; Sanetra & Crozier, 2001; Fernandez-Escudero *et al.*, 2002), including *P. pygmaea* (Trontti *et al.*, 2007). In this species, population-wide sex ratio is highly male biased and females are probably attended by several males in their natal nest. Intranidal mating and female limited dispersal may greatly reduce mating costs. Convenience polyandry could also explain multiple mating in the parasite *P. xene*, although to a lesser extent because numerical sex ratio is female-biased in this species (Aron *et al.*, 1999).

Interestingly, the costs of multiple mating are probably negligible in *P. xene* and *P. pygmaea*. In most ant species, mating takes place outside the natal colony during nuptial flights, thereby exposing the queens to predation risks and elevated energy expenditure (Hölldobler & Wilson, 1990). Transmission of sexual diseases, parasites or pathogens is increased by higher number of matings. Additionally, multiple sperm storage has been shown to negatively affect the immune defence of newly inseminated ant queens (Baer *et al.*, 2006). The survival cost linked to a reduction in immunity may be particularly marked during colony founding, because queens experience greater exposure to pathogens in the absence of grooming workers. These costs are greatly reduced in *Plagiolepis*, because female sexuals mate in the safety of the nest with closely related individuals and they remain in the mother nest after mating or found new colonies dependently. Multiple mating dramatically decreases the relatedness between the workers and the brood they rear and, hence, indirect fitness benefits associated with helping (Hamilton, 1964; Ratnieks, 1988). This cost does not affect the workerless parasite *P. xene*, and reduction in relatedness because of polyandry is cancelled out by the high level of inbreeding in the host *P. pygmaea* (Trontti *et al.*, 2007). Overall, these considerations suggest that there are no or few costs associated with multiple mating in the inquiline parasite *P. xene*, so that polyandry was not or only weakly selected against.

Our results on *P. xene* contrast with those previously reported in the inquiline social parasite *A. insinuator* (Sumner *et al.*, 2004). In this species, only 15% of queens were shown to mate multiply, while this proportion reaches 52% in *P. xene*. Such a difference between both parasites likely stems from profound variations in their breeding system and life history. *Acromyrmex insinuator*

queens take part in nuptial flights, and mating occurs among unrelated individuals. Young mated queens found new colonies independently by entering *A. echinator* host colonies, and these are always headed by a single queen (monogyny) (Bekkevold *et al.*, 1999). It has been argued that the benefit reduction associated with multiple mating in the social parasite no longer counterbalances its costs, thereby justifying a reversion to single mating (Sumner *et al.*, 2004). By contrast, mating in *P. xene* proceeds inside the nest among related individuals, queens found new colonies dependently and colonies are always polygynous (Passera, 1969; Passera *et al.*, 2001; Trontti *et al.*, 2006). This reduces the costs of multiple mating, so that selection for reversion to single mating is probably weak.

In conclusion, our results showing that a large number of queens remain multiply mated in the inquiline parasite *P. xene* do not support the prediction that reversal from multiple to single mating is selected in workerless parasites of polyandrous social insects. They suggest that the extent of reversal depends on the costs and benefits of polyandry, which in turn depend on idiosyncratic differences among species in their life history strategies.

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